- 149** ESTIMATION OF PYRUVATE DEHYDROGENASE (E_1) ACTIVITY IN HUMAN SKELETAL MUSCLE.
Van Laack R, Ruitenbeek W, Trijbels F, Sengers R, Gabreëls F, Kerkhof C, Janssen A.
Depts. of Pediatrics and Neurology, Catholic Univ. of Nijmegen, Nijmegen, The Netherlands.

The pyruvate dehydrogenase complex plays a central role in the pyruvate oxidation. The complex consists of five components of which pyruvate dehydrogenase (E_1) catalyzes the rate limiting step.

Since E_1 has a low activity in human skeletal muscle tissue a sensitive method is needed for diagnostic purposes.

Measurement of $^{14}\text{CO}_2$ -production from [$1-^{14}\text{C}$]pyruvate provides a specific and sensitive assay for measuring E_1 activity in crude extracts. In the E_1 assay an artificial electron acceptor has to be added. We use dichlorophenolindophenol (DCPIP) instead of the often applied ferricyanide. DCPIP shows no inhibitory effects on E_1 activity. E_1 activity appears to be dependent on the cofactors thiaminepyrophosphate and magnesium and is completely inhibited by fluoropyruvate. The method can be applied to frozen or fresh small muscle samples, obtained by needle or open biopsy.

We established a difference in E_1 activity between 600 g supernatant and homogenate of skeletal muscle tissue. The measurement of E_1 activity in supernatant from frozen muscle appeared to be unreliable. Control values in homogenate, 515-2080 nmol.hr $^{-1}$.g $^{-1}$ muscle, are higher or the same as those reported by others.

With the new assay we identified two patients with a E_1 deficiency.

The method appears also suitable for measurement of E_1 activity in chorionic villi.

- 150** ANTENATAL DIAGNOSIS OF MITOCHONDRIOPATHIES.

Ruitenbeek W, Sengers R, Van Laack R, Trijbels F, Bakkeren J, Janssen A, Van Diggelen O.
Dept. of Pediatrics, University of Nijmegen and
Dept. of Clinical Genetics, Erasmus University,
Rotterdam, The Netherlands.

Various abnormalities in mitochondrial enzyme activities have been found, e.g. in myopathies. In only a restricted number of patients these disturbances can be demonstrated in fibroblasts. This restriction is caused by two facts: 1 some defects, found in muscle tissue, are not expressed in cultured fibroblasts and 2 some mitochondrial enzyme activities are hardly measurable in fibroblasts.

Recently, chorionic villi have become important for detecting aberrations in, among others, enzyme activities. Using chorionic villi instead of amnion cells fixes the moment of antenatal diagnosis at an earlier time. In the case of mitochondrial myopathies it is furthermore important that some enzyme activities can be measured in chorionic villi which can not be determined in fibroblasts. Among them are various oxidoreductases of the respiratory chain. Activity of cytochrome oxidase, pyruvate dehydrogenase complex and citrate synthase can be determined in both chorionic villi and fibroblasts.

Control values (in mU/mg protein; mean $\pm$ SD) in chorionic villi:

citrate synthase	49 $\pm$ 18	NADH : O $_2$ oxidoreduct.	5.4 $\pm$ 3.1
cytochrome oxidase	50 $\pm$ 18	NADH : O $_2$ oxidoreduct.	9.1 $\pm$ 5.5
pyruvate DH complex	2.9 $\pm$ 1.3	succinate : cyt c oxid.	4.5 $\pm$ 1.5

The possibilities for antenatal diagnosis of mitochondrial myopathies have now clearly been extended.

- 151** CLINICAL HETEROGENEITY IN GAUCHER DISEASE TYPE 3.
Maaswinkel-Mooy PD, Poorthuis BJHM, Van de Kamp JJP.
Department of Pediatrics, State University Leiden,
The Netherlands.

Gaucher disease (GD) is an autosomal recessive disorder of glycosphingolipid metabolism, resulting from the reduced activity of the lysosomal enzyme β -glucocerebrosidase. Three distinct phenotypes have been delineated, based on the absence or presence and severity of neuropathic involvement: type 1 GD or nonneuronopathic; type 2 or acute neuronopathic, rapidly fatal and type 3 or chronic neuronopathic. The three types must be due to different allelic mutations since no complementation was found after cell fusion. Nevertheless a few publications have appeared describing different types of GD within the same family. Here we report genealogical and clinical data of three patients in one family, two brothers and their cousin, with a wide variation in clinical presentation. The two brothers had severe neurological symptoms from the first year, which place them in type 3; the cousin had no neurological abnormalities and clinically would have been classified as type 1. By immunoblotting techniques of fibroblast homogenates all three were classified as type 3 (or type 2). Our data show that extreme variation in clinical presentation is possible within the same biochemical phenotype, even within one family, which makes classification on clinical grounds alone unreliable.

- 152** EXCESSIVE GLYCEROL EXCRETION IN A MALE INFANT WITH PSYCHOMOTOR RETARDATION, ADRENOCORTICAL INSUFFICIENCY AND EARLY DEATH
Søvik, O., Jellum, E. & Madsen, B.
Department of Pediatrics, University of Bergen
and Institute of Clinical Chemistry,
University of Oslo, Norway

A male, newborn infant (3480g, 54cm) presented with vomiting, slightly dysmorphic facial features, and serum electrolytes compatible with adrenocortical insufficiency. Although the vomiting disappeared after hormone replacement, the infant failed to thrive. Psychomotor retardation was noted, and he gradually developed spasticity. There was "diffuse white matter disease" by cerebral computer tomography. Chromosome analysis showed normal karyotype. Metabolic screening at 6 months of age revealed by sugar chromatography a marked spot with Rf-value 1.5 compared with glucose. By gas chromatography/mass spectrometry this substance was identified as glycerol. The glycerol excretion (1.0-1.5g/24h) was unrelated to diet or drugs. The patient died 12 months old, during a respiratory tract infection. Fibroblasts for glycerol kinase determination were not obtained.

Glyceroluria, with or without demonstrated glycerol kinase deficiency, is an inborn error of metabolism of unknown incidence. The phenotypic expression is variable. Screening may easily be done by urine chromatography (ethyl acetate:pyridine:H $_2$ O, 13:5:4) on paper or thin layer, and visualized by silver nitrate/acetone.

- 153** APPLICATION OF POLYMORPHIC CHROMOSOME 13-SPECIFIC PROBES TO LINKAGE STUDIES IN FAMILIES WITH HEREDITARY RETINOBLASTOMA

Scheffer (1), Tan KEWP (2), Buys CHCM (1)
(1) Dept. Human Genetics, State Univ. Groningen,
(2) Dept. Ophthalmology, State Univ. Utrecht

The isolation of RFLP-revealing DNA sequences from band q14 of chromosome 13 where the retinoblastoma locus has been mapped is a prerequisite for linkage studies in affected families. Until now we have isolated by various approaches nineteen unique chromosome 13-specific DNA sequences from fifteen different loci and subcloned these sequences into pBR329 or pUC9. To find out which sequences derived from 13q14 we constructed a mouse-human cell hybrid lacking 13q14 DNA by carrying out a somatic cell hybridisation between mouse RAG cells and fibroblasts from a patient with retinoblastoma caused by a constitutional deletion of 13q14. We isolated a hybrid cell clone with the deleted chromosome but lacking the normal homologue. Six probes from three different loci did not show hybridisation with DNA from this cell clone. Their putative assignment to 13q14 could be confirmed by means of a mapping panel consisting of hybrid cell lines that we made monosomic for different parts of chromosome 13 by irradiation with X-rays. One of the 13q14 probes detects a low frequency MspI RFLP. A few probes located outside 13q14 revealed high frequency RFLPs. Two 13q14 probes isolated to date have been used to screen a total human cosmid library. Flanking unique human DNA sequences have been isolated from positive cosmids and a search for RFLPs is in progress. Isolated polymorphic probes are now being applied to linkage studies in families with hereditary retinoblastoma.

- 154** LOCALIZATION BY IN SITU HYBRIDIZATION OF DNA PROBES WITH TIGHT LINKAGE TO THE LOCUS FOR THE CYSTIC FIBROSIS MUTATION

van der Hout AH and Buys CHCM
Department of Human Genetics, State University of
Groningen, The Netherlands

Probes 7C22 and pJ3.11 known to be closely linked to the cystic fibrosis locus were made available to us by Prof. Williamson, London. In situ hybridizations were carried out on metaphase preparations of lymphocytes from a patient having an apparent deletion in band q22 of one of the chromosomes 7, but no CF. The site and number of grains were scored for the normal and the deleted homologue. The majority of the grains resulting from hybridization with 7C22 were found in the region q22-qter, with a clustering at the distal part of band 31 and at band 32. For pJ3.11 most of the grains were in the region q31-qter. The normal homologue was more frequently labelled than the deleted one. This difference might be due to random variation, although alternatively structural chromosome aberrations other than deletion might be involved in this patient. In summary, our preliminary results suggest the distal half of the long arm of chromosome 7 to be the location of these probes. Our studies are now being extended to some other probes linked to the cystic fibrosis locus.